Docket No.: NEB-180



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT:

W.E. Jack, et al.

EXAMINER:

SERIAL NO.:

GROUP:

FILED:

FOR:

Use of Site-Specific Nicking Endonucleases To

Create Single-Stranded Regions And

Applications Thereof

Hon. Commissioner of Patents and Trademarks

Sir:

STATEMENTS IN SUPPORT OF FILING AND SUBMISSIONS IN ACCORDANCE WITH 37 C.F.R §§1.821.1-1.825

In accordance with 37 C.F.R. §§1.821-1.825, I hereby state that the contents of the paper and computer-readable copies of the sequence listing submitted in accordance with 37 C.F.R. §1.821(c) and (e), respectively, are the same. I hereby state that the submission, filed in accordance with 37 C.F.R. §1.821(g) does not introduce new matter.

Respectfully submitted,

NEW ENGLAND BIOLABS, INC.

Date: 11/25/00

Gregory D. Williams (Reg. No.: 30901)
Attorney for Applicant

32 Tozer Road Beverly, MA 01915

SEQUENCE LISTING

<110>	Jack, William E. Schildkraut, Ira Menin, Julie F. Greenough, Lucia	
<120>	Use of Site-Specific Nicking Endonucleases to Create Single-Stranded Regions And Applications Thereof	
<130>	NEB-180	
<140>		
<141>		
<160>	51	
<170>	PatentIn Ver. 2.0	
<210>	1	
<211>	40	
<212>	DNA	
<213>	Synthetic oligonucleotide	
<400>	1	
aaatc	aatct aaagtatata ccggtaaact tggtctgaca	40
<210>	2	
<211>	38	
<212>	DNA	
<213>	synthetic oligonucleotide	
<400>	2	
ctage	attag tcagactcta cattcaaata tgtatccg	38
<210>	3	
<211>	38	
<212>	DNA	
<213>	synthetic oligonucleotide	
<400>	3	
gcgct	cgatg tcagactcga gcaaaaggcc agcaaaag	38
<210>	4	
<211>	56	
<212>	DNA	
<213>	synthetic oligonucleotide	

<400> 4	
gagtccgatt gacctaagcg gatactctga cgactcgtag aaaagatcaa aggatc	56
<210> 5	
<211> 51	
<212> DNA	
<213> synthetic oligonucleotide	
<400> 5	
gagtctcaga ctatctggag cgactgactc aaacttggtc tgacagttac c	51
<210> 6	
<211> 40	
<212> DNA	
<213> synthetic oligonucleotide	
<400> 6	
gtaaatatcg gactctacaa tcaaatatgt atccgctcat	40
<210> 7	
<211> 82	
<212> DNA	
<213> synthetic oligonucleotide	
<400> 7	
gatcgagtct gacatcgagc gcctagcatt agtcagactc gatatcgagt ctcagcctgt	60
tagcgatggt acatgacgac tc	82
<210> 8	
<211> 82	
<212> DNA	
<213> synthetic oligonucleotide	
<400> 8	
ctaggagtcg tcatgtacca tcgctaacag gctgagactc gatatcgagt ctgactaatg	60
ctaggcgctc gatgtcagac tc	82
<210> 9	
<211> 22	
<212> DNA	
<213> synthetic oligonucleotide	
<400> 9	
catgtctaga ctgcagagat ct	22
<210> 10	
<211> 18	
<212> DNA	

<213> synthetic oligonucleotide	
<400> 10	
agatctctgc agtctaga	18
<210> 11	
<211> 21	
<212> DNA	
<213> synthetic oligonucleotide	
<400> 11	
tacattcaaa tatgtatccg c	21
<210> 12	
<211> 21	
<212> DNA	
<213> synthetic oligonucleotide	
<400> 12	
taaacttggt ctgacagtta c	21
<210> 13	
<211> 54	
<212> DNA	
<213> synthetic oligonucleotide	
<400> 13	
gagtatccgc ttaggtcaat cggactcgga ccggatatca catgtgagtc gtca	54
<210> 14	
<211> 54	
<212> DNA	
<213> synthetic oligonucleotide	
<400> 14	
cctgttagcg atggtacatg acgactcaca tgtgatatcc ggtccgagtc cgat	54
<210> 15	
<211> 10	
<212> DNA	
<213> N.BstNBI Recognition Sequence	
<220>	
<pre><223> N indicates any base (subject to the normal rules</pre>	
of base pairing between the strands).	
<400> 15	
gagtcnnnnn	10

<210>	16	
<211>	18	
<212>	DNA	
<213>	Artificial Sequence	
<220>	Denoviation of Dutificial Grands mbassation	
<223>	Description of Artificial Sequence: Theoretical	
	sequences - all randomly generated	
<400>	16	
		18
909000		
<210>	17	
<211>	18	
<212>	DNA	
<213>	Artificial Sequence	
<220>		
<223>	Description of Artificial Sequence: Theoretical	
	sequences - all randomly generated	
<400>	- '	18
gcgtto	caaac ccagatgt	Τ0
<210>	18	
<211>	18	
<212>	DNA	
<213>	Artificial Sequence	
<220>		
<223>	Description of Artificial Sequence: Theoretical	
	sequences - all randomly generated	
	10	
<400>		18
agetg	ctcta agccgcaa	10
<210>	19	
<211>		
<212>		
<213>	Artificial Sequence	
	-	
<220>		
<223>	Description of Artificial Sequence: Theoretical	
	sequences - all randomly generated	
<400>		1.0
tgtga	acacc tcgtaacg	18

<210> 2	20	
<211> 1	18	
<212> [DNA	
<213> A	Artificial Sequence	
<220>		
<223> I	Description of Artificial Sequence: Theoretical	
S	sequences - all randomly generated	
<400> 2	20	
ttcccaa	agca catgggat	18
<210> 2	- 	
<211> 1		
<212> I		
<213> P	Artificial Sequence	
<220>		
	Description of Artificial Sequence: Theoretical	
٤	sequences - all randomly generated	
<400> 2		
tctccaa	agca cagtgagt	18
-010- 0		
<210> 2		
<211> 1		
<212> I		
<213> P	Artificial Sequence	
<220>		
	Description of Artificial Sequence: Theoretical	
	sequences - all randomly generated	
•	sequences - all randomly generated	
<400> 2	22	
		18
cgacco	aago gagtacto	
<210> 2	23	
<211>		
<212> I		
	Artificial Sequence	
-220, 1		
<220>		
	Description of Artificial Sequence: Theoretical	
	sequences - all randomly generated	
<400>	23	
tgactca	aagc ggatactc	18

<210> 24	
<211> 18	
<212> DNA	
<213> Artificial Sequence	
<220>	
<223> Description of Artificial Sequence: T	Pheoretical
sequences - all randomly generated	
<400> 24	
tgcatcaage ggatacte	18
tycatcaage ggatacte	10
<210> 25	
<211> 18	
<212> DNA	
<213> Artificial Sequence	
-	
<220>	
<223> Description of Artificial Sequence:	Theoretical
sequence - randomly generated	
<400> 25	
actgagcgcc atgcatta	18
010 05	
<210> 26	
<211> 18	
<212> DNA	
<213> Artificial Sequence	
<220>	
<pre><223> Description of Artificial Sequence:</pre>	Theoretical
sequence - randomly generated	
<400> 26	
actgagcgcc agtcatta	18
<210> 27	
<211> 18	
<212> DNA	
<213> Artificial Sequence	
.220.	
<220>	Theoretics?
<pre><223> Description of Artificial Sequence: company = randomly generated</pre>	INCOLECTOAL
sequence - randomly generated	
<400> 27	
ategagegee atgeatta	18

<210>	28	
<211>	18	
<212>	DNA	
<213>	Artificial Sequence	
<220>	The state of the s	
<223>	Description of Artificial Sequence: Theoretical	
	sequence - randomly generated	
<400>	28	
	gegee tageatta	18
acogas	,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	
<210>	29	
<211>	18	
<212>	DNA	
<213>	Artificial Sequence	
<220>		
<223>	Description of Artificial Sequence: Theoretical	
	sequence - randomly generated	
<400>		1.0
tgtac	catcg ctaacagg	18
<210>	3.0	
<211>		
<212>		
	Artificial Sequence	
<220>		
<223>	Description of Artificial Sequence: Theoretical	
	sequence - implemented via the synthetic	
	oligonucleotide, but never existed as independent	
	entity	
<400>		26
gagtc	tgaca tcgagcgcct agcattagtc agactc	36
<210>	31	
<211>		
<212>		
	Artificial Sequence	
	- -	
<220>		
<223>	Description of Artificial Sequence: Theoretical	
	sequence - implemented via the synthetic	
	oligonucleotide, but never existed as independent	

entity.

<400> 31	
gagtccgatt gacctaagcg gatactctga cgactc	36
<210> 32	
<211> 36	
<212> DNA	
<213> Artificial Sequence	
<220>	
<223> Description of Artificial Sequence: Theoretical	
sequence - implemented via the synthetic	
oligonucleotide, but never existed as independent	
entity	
<400> 32	
gagteteage etgttagega tggtacatga egaete	36
gagteteage etgetagega tggtacatga egacte	30
<210> 33	
<211> 50	
<212> DNA	
<213> synthetic oligonucleotide	
<220>	
<223> N/A	
<400> 33	
gagtcagctc aatgttgcca gtcaggactc gtagaaaaga tcaaaggatc	50
gagecagete aatgetgeed geologiaete geologiaaaga eelaaaggaee	
<210> 34	
<211> 35	
<212> DNA	
<213> synthetic oligonucleotide	
<220>	
<223> N/A	
<400> 34	
gggccacgta gactcgagca aaaggccagc aaaag	35
<210> 35	
<211> 12	
<212> DNA	
<213> Artificial Sequence	
~220\	
<pre><220> <223> Description of Artificial Sequence: Theoretical</pre>	

sequence - randomly generated

<400> 35 gtagttacgc ca	12
×21.0× 2.6	
<210> 36 <211> 12	
<211> 12 <212> DNA	
<213> Artificial Sequence	
VZIJV AICITICIAI BOQUENCO	
<220>	
<223> Description of Artificial Sequence: Theoretical	
sequence - randomly generated	
<400> 36	
caatgttgcc ag	12
010 05	
<210> 37	
<211> 12 <212> DNA	
<213> Artificial Sequence	
\213> Altilitial bequence	
<220>	
<223> Description of Artificial Sequence: Theoretical	
sequence - randomly generated	
<400> 37	
tctcaatgag gc	12
010 20	
<210> 38	
<211> 12 <212> DNA	
<213> Artificial Sequence	
VZIJ/ AICITICIAI Bequeñoc	
<220>	
<223> Description of Artificial Sequence: Theoretical	
sequence - randomly generated	
<400> 38	
agcgagcctt ta	12
-210- 20	
<210> 39 <211> 12	
<211> 12 <212> DNA	
<213> Artificial Sequence	
<220>	
<223> Description of Artificial Sequence: Theoretical	

sequence - randomly generated

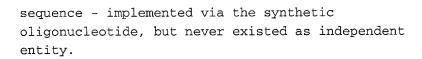
<400> 39 tgatcgagac ct	12
<210> 40 <211> 12	
<212> DNA	
<213> Artificial Sequence	
<220>	
<223> Description of Artificial Sequence: Theoretical sequence - randomly generated	
<400> 40	
tctgcggata ac	12
<210> 41	
<211> 12	
<212> DNA	
<213> Artificial Sequence	
<220>	
<pre><223> Description of Artificial Sequence: Theoretical sequence - randomly generated</pre>	
<400> 41	
tatgcagcgc at	12
<210> 42	
<211> 12	
<212> DNA	
<213> Artificial Sequence	
<220>	
<223> Description of Artificial Sequence: Theoretical	
sequence - randomly generated	
<400> 42	
	12
<400> 42 gatcgaacgt tc <210> 43	12
<400> 42 gatcgaacgt tc	12
<400> 42 gatcgaacgt tc <210> 43 <211> 12 <212> DNA	12
<400> 42 gatcgaacgt tc <210> 43 <211> 12	12
<400> 42 gatcgaacgt tc <210> 43 <211> 12 <212> DNA	12





sequence - randomly generated

<400> 43	
11007 13	
aaatttgggc cc	12
<210> 44	
<211> 12	
<212> DNA	
<213> Artificial Sequence	
<220>	
<223> Description of Artificial Sequence: Theoretical	
sequence - randomly generated	
<400> 44	
actatctgga gc	12
<210> 45	
<211> 12	
<212> DNA	
<213> Artificial Sequence	
12137 III dilicial boquesso	
<220>	
<223> Description of Artificial Sequence: Theoretical	
sequence - randomly generated	
\$644.600	
<400> 45	
aggcgacatt tc	12
<210> 46	
<211> 12	
<212> DNA	
<213> Artificial Sequence	
varov incontrolar poquonos	
<220>	
<223> Description of Artificial Sequence: Theoretical	
<223> Description of Artificial Sequence: Theoretical sequence - randomly generated	
<223> Description of Artificial Sequence: Theoretical sequence - randomly generated <400> 46	12
<223> Description of Artificial Sequence: Theoretical sequence - randomly generated	12
<223> Description of Artificial Sequence: Theoretical sequence - randomly generated <400> 46 atttacgggc ca	12
<223> Description of Artificial Sequence: Theoretical sequence - randomly generated <400> 46 atttacgggc ca <210> 47	12
<223> Description of Artificial Sequence: Theoretical sequence - randomly generated <400> 46 atttacgggc ca <210> 47 <211> 30	12
<pre><223> Description of Artificial Sequence: Theoretical sequence - randomly generated <400> 46 atttacgggc ca <210> 47 <211> 30 <212> DNA</pre>	12
<223> Description of Artificial Sequence: Theoretical sequence - randomly generated <400> 46 atttacgggc ca <210> 47 <211> 30	12
<pre><223> Description of Artificial Sequence: Theoretical</pre>	12
<pre><223> Description of Artificial Sequence: Theoretical sequence - randomly generated <400> 46 atttacgggc ca <210> 47 <211> 30 <212> DNA</pre>	12



<400> 47 gagtcagctc aatgttgcca gtcaggactc	30
<210> 48 <211> 30 <212> DNA <213> Artificial Sequence	
<pre><220> <223> Description of Artificial Sequence: Theoretical sequence -implemented via the synthetic oligonucleotide, but never existed as independent entity.</pre>	
<400> 48	
gagtccgata tttacgggcc acgtagactc	30
<210> 49 <211> 30 <212> DNA <213> Artificial Sequence	
<220> <223> Description of Artificial Sequence: Theoretical sequence - implemented via the synthetic oligonucleotide, but never existed as independent entity.	
<400> 49	
gagteteaga etatetggag egaetgaete	30
<210> 50 <211> 48 <212> DNA <213> synthetic oligonucleotide	
<220>	
<223> Description of Artificial Sequence:N/A	
<400> 50 ctggcaacat tgatcggact cggaccggat atcacatgtg agtcgtca	48
<210> 51 <211> 48	

<212>	DNA	
<213>	synthetic	oligonucleotide

<220> <223> Description of Artificial Sequence:N/A

<400> 51
gctccagata gttgacgact cacatgtgat atccggtccg agtccgat

48